TRANSLATION

COACERVATES AND FERMENTS ALBUMIN-CARBOHYDRATE COACERVATES AND ALPHA-AMYLASES

By

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Coacervates and Ferments Albumin-Carbohydrate Coacervates and Alpha-Amylases

By

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In investigations [3,5] were obtained complex coacervates and their properties have been investigated in the given report, together with the obtainment and investigation of complex albumina-carbohydrate coacervates as such* it was of interest to include ferments [3.] In literature were not found investigations with the inclusion of ferments in coacervates. In role of ferment was taken alph-amylases, and the coacervate was obtained from protamine sulfate, gelatin and soluble starch.

Alpha-amylase was separated from cultured liquid of a thermophiliv variant of Clostidium paterianum, growing at a temperature of + 60°. The optimum for the given effect of the compound is a temperature of + 60° and pH %.5 - 5/9. But alpha-amlyase is active also at temperatures of from + 45 to + 90° and at pH of from 5.0 to 9.0. Therefore to study its effect in the coacervate were obtained coacervate droplets, consisting of dissolved starch, protamine sulfate and gelatin. Such droplets are formed at pH of from 5.6 to 8.4 and temperature of

The method of studying the effect of aplha-amylase in such coacervates consisted of the following:

To 0.4 ml of 1% solution of soluble starch were added 0.4 ml of 0.5% protamine sulfate solution, 1.2 ml of 0.67% gelating solution** and 0.1 ml alpha-

* Under coacervate is undersoood a system of coacervate droplets or a layer enriched with colloidal substances and equilibrium liquid surrounding it.
**. Soluble Kahlbaum starch. Protamine sulfate [1, 2, 6,] 8 was obtained and purified by double deposition by the Kossel [7] method from lactates of freshly frozen far eastern sickle (Onchorhynchus gorbusha). The compound represents a white amorphous powder; the amount of found nitrogen was 18.71% sulfur 5.71% or sulfuric acid 17.49%.

amylase, containing 0.2 mg of ferment. All solutions were first heated to + 50° and mixed in indicated sequence. The mixture was then alkalized with 0.01 n of NaOH solution to pH 7.0, at which the coacervate droplets were formed *** pH 7.0 was found to be most approaching, because in a more aciduous zone too fine coacervate droplets are being formed. Furthermore, at a higher alkaline reaction the iodine does not give a characteristic coloring with starch and products of its decomposition-dextrins, and in the given report about the effect of alpha-amylase on starch in coacervate droplets an opinion was made by the various colors of these droplets in an iodine solution. After formation of coacervate droplets the coacervate was kept at + 50° and within certain time intervals, counted by a stopwatch, samples were taken on subject quartz glasses and colored with 0.02 n J_2 in KJ. Within the first 10 minutes samples were taken each 30 sec, and then within every minute.

Such colored coacervate droplets were photographed with the aid of an MUF-2 unit and a chromoscope on a chromatic film. In fig.1.in the first photo are shown coacervates, containing starch, the second one shows coacervates, in which the starch has decomposed to a stage of amylodextrin, on the third one - to erythrodextrins and on the fourth one - approaching achrodextrins.

Simultaneously were made control determinations with inactivated boiling alpha-amylase . The color of the coacervate droplets in this case was bluish-violet and stayed that way for the entire time of experimentation.

*** In all instances, where we speak about coacervate droplets, a proper check was made of their presence under the microscope at an 80 X magnification.

** cont. Its isoelectric point lies at pH 8.9; the measurements were made with a calomel and glass electrodes on an LP-4 potentiometer in a 0.25% solution (to the solution in test tubes was added 0.02 n NaOH to clear turbidity-moment of maximum turbidity was taken as the isoelectric point).a 0.67% gelatin solution did not absorb ultraviolet rays in MUF-2 when λ = 250 - 280 mμ and over/. The mentioned substances should be pure, especially the soluble starch.

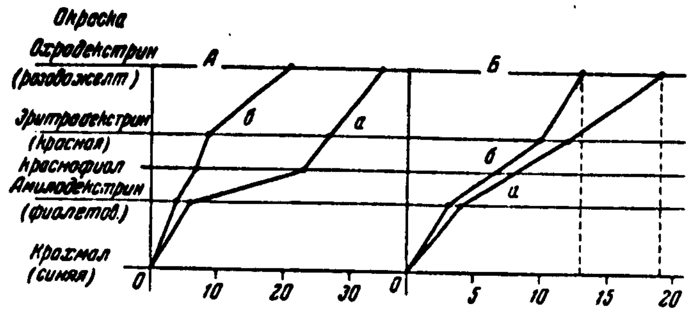

Fig.2.Rate of decomposition of starch under the effect of alpha-amylase. A: a-alpha-amylase + coacervate, pH 7.0; b- alpha-amylase + soluble starch and water pH 7.1. B: a- alpha-amylase + mixture of (gelatin + protamine + soluble starch), pH 5.5; b - alpha-amylase + soluble starch and water, pH 5.2
1- color; achrodextrin (pinkish-yellow). 2- erythrodextrin/(red) red violet; Amilo-dextrin (violet). 3- starch (blue).

Parallel checks were made on the effect of alpha-amylase on soluble starch as such. When these experiments were set up to protect the volumetric ratios, existing in the coacervate, instead of gelatin and protamine solutions was taken a corresponding amount of water (see fig.2A, curve b).

A comparison of curves a and b in fig.2A shows that in coacervated the starch decomposes for a longer period, than in an ordinary solution.

It is possible, that the very components of the coacervate - gelatin and protamine - reduce the activity of the ferment. To check the effect of the ferment on soluble starch in the presence of a gelatin and protamine solutions at pH 7.0,unfortunately, is impossible,because at this pH takes place the formation of coacervate droplets. Consequently to quantitatively determine the rate of decomposition of soluble starch with the participation of alpha -amylase in the coacervates and in ordinary solutions at one and the same pH was impossible. But to check this assumption was determined the activity of alpha-amylase in mixture, consisting of the very same solutions and in very same ratio, as when studying the activity of alpha-amylase in coacervates, but at pH 5.2 - 5.5 and in pure solution of soluble starch. At indicated pH coacervates are not being formed, the activity of the alpha-amylase compound is higher, that at pH 7.0. Experimental results are presented by

curves a and b in fig.2B.

It is evident from these data that gelatin and protamine slow down decomposition of starch at pH 5.2 - 5.5.

In coacervate droplets gelatin and protamine are concentrated. The viscosity of these droplets is much higher, than in a simple mixture of gelatin/protamine solutions.

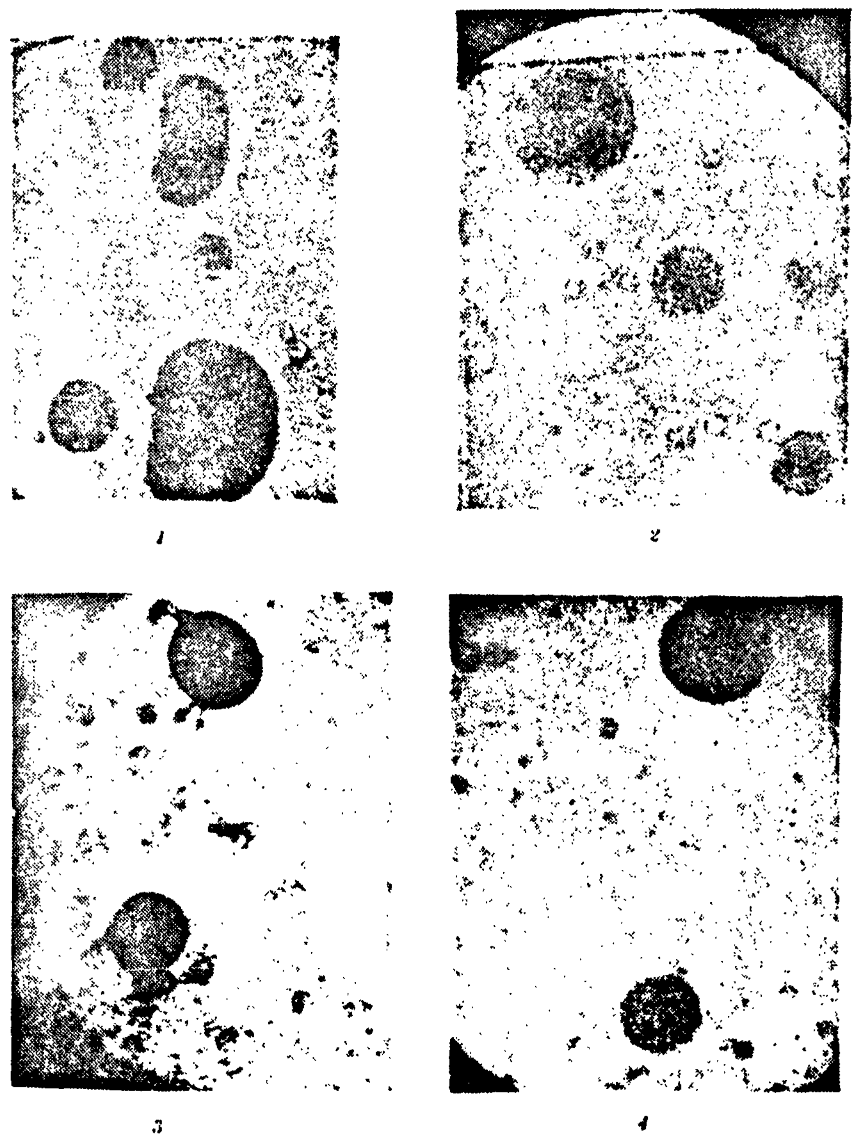

Fig.1.

Consequenly, it is perfectly possible in the given case to raise the adsorbability of the droplets and reduce the rate of motion of the substances in them; and fermentation processes (especially decomposition of starch) in coacervate droplets are also slower, than in ordinary solutions. This investigation appears to be the first effort to complicate coacervates by including in them ferments and by studying their effect in such systems.

Submitted May 17, 1955

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